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論文題目	Human Atrial Natriuretic Peptide in Cold Storage of Donation after Circulatory Death Rat Livers: An Old but New Agent for Protecting Vascular Endothelia? （ヒト心房性ナトリウム利尿ペプチド (hANP)の保存液添加は、心停止後摘出肝臓の血管内皮保護効果を介して冷虚血/温再灌流傷害を軽減する）		

（論文内容の要旨）

Backgrounds and Purposes

Current critical shortage of donor organs has increased the use of donation after circulatory death (DCD) livers for transplantation, despite higher risk for primary non-function. Although much attention has recently paid to machine perfusion (MP) preservation, the necessity for special equipments, human resources, and high medical costs have hampered its widespread clinical translation. Human atrial natriuretic peptide (hANP) is an endogenous, cardiovascular hormone that possesses protective action to vascular endothelia. This study was thus design to clarify the therapeutic potential of hANP in static cold storage (CS) of DCD livers, the gold standard method for organ preservation.

Materials and Methods

Male Wistar rats were exposed to bilateral phrenotomy and subsequent cardiac arrest *in situ*, remained untouched thereafter for 30 minutes. Livers were then retrieved and cold-preserved in UW (University of Wisconsin) solution for 6 hours with or without 2.5 μg/mL of hANP supplementation. Functional and morphological integrity of the livers was evaluated by oxygenated *ex vivo* reperfusion at 37°C.

Results

hANP supplementation resulted in significant reduction of portal venous pressure (12.2 ± 0.5 *vs*. 22.5 ± 3.5 mmHg, P <0.001). As the underlying mechanisms, hANP significantly increased tissue adenosine concentration (P =0.008), followed by significant up-regulation of endothelial nitric oxide synthase (eNOS) and endothelin-1 down-regulation than in the controls (P =0.01 and P =0.004, respectively). Consequently, hANP significantly decreased transaminase release (ALT: 134.2 ± 14.2 vs. 223.3 ± 31.5 U/L, P <0.001), and increased bile production (96.2 ± 18.2 *vs*. 36.2 ± 15.2 μL/g-liver/hour, P <0.001). Morphologically, hepatocytes and sinusoidal endothelia were both better maintained by hANP (P =0.021). Electron microscopy also revealed that sinusoidal ultra-structures and microvilli formation in bile canaliculi were both better-preserved by hANP supplementation. Silver staining also demonstrated that hANP significantly preserved reticulin fibers in Disse space (P =0.017), representing significant protection of sinusoidal frameworks and architectures.

Discussion

Supplementation of hANP during CS significantly protected DCD liver grafts from warm and cold ischemia/reperfusion injury. Given its simplicity, cost-effectiveness, and unobtainable safety, hANP supplementation during CS may be a novel therapeutic option for DCD liver grafts, which is easily applicable worldwide, even though highly-equipped MP devises are not available.

（論文審査の結果の要旨）

肝移植医療においてドナー不足は解決されるべき課題であり、心停止後摘出臓器（DCD）の移植利用が世界的関心事である。今回 申請者は、ラット DCD 肝と体外灌流装置を用いて、ヒト心房性ナトリウム利尿ペプチド（hANP）の臓器保存液への添加による、冷虚血/温再灌流障害の抑制効果について検討を行った。

hANP 添加群において 温再灌流中の門脈灌流圧は有意に低下し、その機序として肝組織中 adenosine 濃度の上昇と，引き続く endothelial nitric oxide synthase 発現増加、endothelin-1 発現抑制があることを示した。一方、臓器保護効果については、hANP 群における温再灌流中の肝酵素逸脱抑制、胆汁産生量の増加を示した。また電子顕微鏡像、鍍銀染色標本像により、hANP 添加による類洞壁微細構造 及び 毛細胆管微絨毛構造の健常性維持効果を示した。

以上より、hANP の臓器保存液添加が、微小循環障害保護機構を介して DCD 肝の冷虚血/温再灌流障害を抑制することを実証し、DCD 肝の移植利用に向けた新たな治療戦略の一つとなる可能性が示された。

以上の研究は、肝移植における虚血再灌流障害の機序解明と、hANP による DCD 肝の新規臓器保存法の開発に貢献し、肝移植成績の改善に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成３１年２月４日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。